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Effects of dietary *Syzygium aromaticum* leaf meal supplementation on blood profile and oxidative status of laying hens

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Abstract

Background: The study examined the effects of dietary supplementation of *Syzygium aromaticum* leaf meal (SLM) on the haematology, serum biochemistry and antioxidative status of laying hens.

Results: A total of 150 20-week-old Isa brown birds were randomly assigned to three experimental diets: Diet 1/control (no supplementation), Diet 2 (0.25% SLM) and Diet 3 (0.50% SLM). Each experimental diet was further divided into five (5) replicates and comprised 10 birds per replicate, that is, 50 birds per experimental diet. The birds were fed with layers mash for 32 weeks of the feeding trial. Blood samples collected in the 32nd week were subjected to haematological and serum biochemical analyses. The dietary supplementation of 0.25% and 0.50% SLM did not significantly ($P > 0.05$) affect the haematological parameters observed in the layer birds which indicated that SLM did not render the birds anaemic nor compromise their immunity. Likewise, the result of the serum protein (total protein, albumin, globulin and albumin: globulin), serum enzymes (alanine and aspartate aminotransferases) and serum metabolites (creatinine, uric acid and glucose) were not affected ($P > 0.05$) by the dietary treatment except for the concentration of serum catalase and glutathione peroxidase which was statistically higher ($P < 0.05$) in layers fed with diets supplemented with SLM compared to the control group, while the serum superoxide dismutase concentration of laying hens was not significantly influenced ($P > 0.05$).

Conclusion: The supplementation of SLM at 0.25% and 0.50% in layers diet can be referred to as a safe additive as the blood indices of birds were not adversely affected. Also, the liver functions which were investigated through the serum enzymes were not impaired and oxidative stress is not triggered in the birds.

Keywords: *Syzygium aromaticum*, Haematology, Serum protein, Serum metabolites, Serum enzymes, Layers, Supplementation

Background

In recent years, there has been a hike in the demand for antibiotic-free products despite the high feed cost in the poultry industry. The residual effects of antibiotics on man had led to the investigation and utilization of herbs, spices and their extracts as natural feed additives in poultry diets due to their inherent multi-bioactive properties (Adu et al. 2020). The supplementation of phytogenics in poultry diets is becoming more popular as it is cheap and readily available, less toxic, rich in nutrients and useful in improving the health of animals and consumers (Dhama et al. 2015). Phytogenics enhance amino acid availability and increase performance in body weight of the birds, carcass traits, and health status and limit the adverse effects of antioxidative stress and ammonia emissions in birds (King 2017; Valenzuela-Grijalva et al. 2017; Oloruntola et al. 2018) as well as lower cholesterol

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content thereby improving meat quality (Oloruntola et al. 2018, 2019).

Syzygium aromaticum (clove) is an aromatic plant commonly used as a spice. It is an evergreen plant available all year round with different harvest seasons in different countries (Yun 2018). Aside from vitamins A, K, B6, B1 and C present in *S. aromaticum* (Dorman and Deans 2000), it contains numerous biologically active compounds such as eugenol (72–90%), eugenol acetate, β-caryophyllene, flavonoids and triterpenoids (Bhowmik et al. 2012; Jimoh et al. 2017). It has been well reported for its antimicrobial properties (Wang and Kim 2011) and immune-stimulating properties which have positive effects on the growth performance and health of poultry (Chowdhury et al. 2018; Kunnumakkara et al. 2018, Al-Mufarrej et al. 2019). Clove and its essential oil are highly relevant in poultry to improve growth performance by enhancing the intestinal microbiota population (Mohammadi et al. 2014). The supplementation of *S. aromaticum* powder at 1 g of clove/kg diet of broiler chickens was reported to boost immunity and antioxidant activity in the broiler (Mahrous et al. 2017). Adu et al. (2020) further reported that the supplementation of *Myristica fragrans* seed meal and *S. aromaticum* leaf meal at 0.25% in a broiler's diet improved body weight gain and endogenous antioxidant enzymes, maintain carcass traits, organ weight and gut microflora and reduced meat cholesterol and lipid oxidation in broiler chickens. The use of clove at a level greater than 2% was reported by (Al-Mufarrej et al. 2019) to have no adverse effects on liver functions and immunity although the palatability of the feed was negatively affected resulting in reduced growth performance in the broiler chickens. Several studies have investigated the effect of clove on various growth and physiological parameters in broiler chickens although there is a dearth of information on the efficacy of dietary clove supplementation in laying hens. Therefore, the objective of this study was to evaluate the effects of dietary supplementation of *S. aromaticum* leaf meal (SLM) on the growth performance, haematology, serum biochemistry and antioxidative status of laying hens.

Methods

Collection and processing of plant samples

Dry *S. aromaticum* leaf was sourced from the local markets in SouthWestern, Nigeria. It was ground with the aid of a hammer mill to about 100 µm to produce *S. aromaticum* leaf meal (SLM).

Animal management and experimental design

The layer's mash fed to the birds was formulated according to (NRC 1994) nutritional recommendations (Table 1). The basal diet was divided into three equal parts and named; diets 1–3. Diet 1 (control) has 0% supplementation, diet 2 contains 0.25% SLM and diet 3 contains 0.50% SLM. One hundred and fifty 20-week-old Isa brown birds were procured from a reputable source and were randomly assigned to three experimental diets (50 birds/experimental diet) in a completely randomized design with five replicates per treatment. Ten (10) birds were assigned to each replicate.

Table 1 Composition of Layer's experimental diet

Ingredients	% Composition
Maize	50.00
Rice bran	8.00
Palm kernel cake	2.60
Groundnut cake	15.00
Soybean meal	15.00
Fishmeal	5.00
Limestone	1.50
Premix	0.30
Di-calcium phosphate	1.00
Methionine	0.20
Lysine	0.10
Salt	0.30
Total	100

Slaughtering procedures and blood sample collection

All experimental procedures were approved by the Animal Welfare Committee of the Federal University of Technology, Akure, after the due proposal has been presented and approved. At the end of the 32nd week of lay, three experimental birds per replicate were randomly selected, labelled and humanely killed as described by (Oloruntola et al. 2019). The jugular veins at the birds' neck region were cut with a sterilized stainless knife. Blood samples were collected into Ethylenediamine tetraacetic acid (EDTA) bottles for haematological studies and plain bottles for serum antioxidant enzymes and serum enzymes analyses. The blood samples in the plain bottles were spined and their sera were harvested into other plain well-labelled bottles and frozen at – 20 °C before analysis. The haematological indices were determined within 120 min post-collection (Shastry 1983). The concentrations of serum enzymes were analysed on a Reflectron ®Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany) using kits. Also, the serum antioxidant enzymes i.e. superoxide dismutase (Misra and Fridovich., 1972), glutathione peroxidase (Rotruck et al. 1973) and catalase (Aebi 1974) were determined. On completion of the study, the birds are sold off after few weeks as spent birds.

Data analysis

The statistical model used for the experiment:

$$Yij = \mu + Ci + eij$$

where μ is the general mean, Ci is the effect of treatment ($i = 1,2,3$), it is the random error associated with Yij observation.

The experiment was subjected to completely randomized design procedures of SAS (2008, version 9.2). The means were compared using the Duncan Multiple Range Test of the same software where the analysis of variance indicated a significant treatment effect at a 5% level of significance.

Results

Haematological indices of laying hen

The haematological profile of the laying birds fed with diets supplemented with *S. aromaticum* leaf meal (SLM) is presented in Table 2. The erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell (RBC), haemoglobin concentration (Hb), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) and mean cell haemoglobin (MCH) were not significantly ($P > 0.05$) affected by SLM when compared with the control.

White blood cell differential of laying hens

Table 3 shows the white blood cell differential of laying hens fed with a diet containing SLM. The results of the lymphocytes, heterophils, monocytes, basophils and eosinophils were not significantly ($P > 0.05$) influenced by SLM compared with the control.

Serum protein, enzymes and metabolites of laying hens

The serum proteins, enzymes and metabolites of laying hens fed with dietary SLM are shown in Table 4. The results of the serum proteins (total protein, albumin, globulin and albumin: globulin), serum enzymes (alanine aminotransferase and aspartate aminotransferase) and serum metabolites (creatinine, uric acid and glucose) were not affected ($P > 0.05$) by the dietary treatment. The concentrations of serum catalase and glutathione peroxidase were significantly ($P < 0.05$) higher in layers fed with diets supplemented with SLM, compared to the control group, while the dietary SLM had no significant effect ($P > 0.05$) on serum superoxide dismutase concentration of laying hens.

Table 2 Haematology of laying hens fed diets supplemented and *Syzygium aromaticum* leaf meal

Variables	Control	Clove (% diet)		*P*-Value
	0.00%	0.25%	0.50%	
ESR (mm)	8.00 ± 3.06	8.33 ± 0.33	8.67 ± 1.20	0.81
PCV (%)	22.33 ± 3.33	23.00 ± 1.15	21.67 ± 0.67	0.21
RBC ($\times 10^6 mm^3$)	26.97 ± 3.27	25.43 ± 2.60	25.77 ± 1.87	0.11
Hb (g/dl)	7.10 ± 1.10	7.67 ± 0.38	7.23 ± 0.23	0.21
MCHC (g/dl)	33.30 ± 0.03	33.34 ± 0.08	33.38 ± 0.05	0.63
MCV (fl)	10.14 ± 0.10	11.06 ± 0.92	11.19 ± 0.88	0.14
MCH (pg)	3.38 ± 0.03S	3.42 ± 0.31	3.20 ± 0.29	0.13

Table 3 White blood cell differentials of laying hens fed diets *Syzygium aromaticum* leaf meal

Variables	Control	Clove (% diet)		*P*-Value
	0.00%	0.25%	0.50%	
Lymphocytes (%)	62.33 ± 0.67	62.53 ± 0.88	62.00 ± 0.58	0.45
Heterophils (%)	20.67 ± 1.45	19.53 ± 1.76	19.67 ± 1.45	0.37
Monocytes (%)	12.67 ± 1.20	12.33 ± 0.88	12.67 ± 1.45	0.35
Basophils (%)	3.33 ± 0.33	3.50 ± 0.00	3.67 ± 0.33	0.30
Eosinophils (%)	2.00 ± 0.10	2.00 ± 0.12	2.00 ± 0.08	0.10

Discussion

Most medicinal herbs or spices, especially clove possess immune-boosting and anti-viral properties (Sharma and Choudhary 2017). The erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cells (RBC), haemoglobin (Hb), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) and mean cell haemoglobin (MCH) of the laying hens were not significantly affected by the dietary supplementation of SLM compared to the control group. This observation agrees with the findings of (Adu et al. 2020) who reported no significant differences in PCV, RBC, Hb and MCHC of broiler chickens fed clove. The findings also correlated with the research work carried out by (Tang et al. 2017) who stated that the use of prebiotics and probiotics had no significant effects on haematological parameters (RBC, Hb, PCV, MCV and MCHC), as natural feed additives, as reported by (Oloruntola et al. 2019), support normal haemopoietic processes of the laying hen.

Likewise, no significant differences were observed among the white blood cell differentials of the birds fed the diets supplemented with clove and the control diet. The various white blood parameters (lymphocytes, heterophils, monocytes, basophils and eosinophils) were within the normal range of haematological values for chickens which indicated that SLM had no harmful effects on the immunity of the laying hen as reported by (Panda et al. 2008). Also, the non-significant effects of SLM on the laying hen could be attributed to the

Table 4 Serum biochemistry of laying hens fed diets supplemented with *S. aromaticum* leaf meal

Variables	Control	Clove (% diet)		*P*-Value
	0.00%	0.25%	0.50%	
Serum protein (g/dl)				
Total protein	6.45 ± 0.25	6.40 ± 0.15	6.30 ± 0.17	0.94
Albumin	3.25 ± 0.05	3.10 ± 0.07	3.50 ± 0.09	0.24
Globulin	3.20 ± 0.20	3.30 ± 0.13	2.98 ± 0.17	0.59
Albumin: globulin	1.02 ± 0.20	0.94 ± 0.06	1.25 ± 0.12	0.28
Serum metabolites				
Alanine transferase (IU/L)	7.45 ± 0.05	8.00 ± 0.14	7.80 ± 0.05	0.15
Aspartate transferase (IU/L)	170.00 ± 3.00	172.00 ± 2.01	173.00 ± 2.50	0.61
Uric acid (mg/dl)	10.10 ± 0.02	11.00 ± 0.04	10.70 ± 0.01[a]	0.03
Creatinine(µmol/l)	159.70 ± 13.40	154.90 ± 6.40	155.60 ± 7.63	0.85
Glucose (mg/dl)	133.23 ± 10.75	130.28 ± 4.75	135.48 ± 5.32	0.92
Serum antioxidative enzymes				
Catalase (mM/ml/min)	5.51 ± 1.11[b]	9.54 ± 1.09[a]	10.16 ± 0.93[a]	0.04
Glutathione Peroxidase (µg/g)	79.08 ± 37.55[b]	89.54 ± 0.43[a]	86.25 ± 0.25[a]	0.03
Superoxide Dismutase (%)	76.58 ± 37.55	75.004 ± 0.43	76.75 ± 0.25	0.07

a, b are superscripts that indicates the means across the row were significantly different ($P < 0.05$)

immune-stimulatory properties of the clove (Chowdhury et al. 2018; Kunnumakkara et al. 2018).

Findings from this study revealed no significant effects of SLM on the serum proteins and serum metabolites which indicated that clove does not pose any health challenges to the laying birds, especially as it relates to the liver (Oboh and Akindahunsi 2005). These research findings align with the experiment carried out by (Al-Mufarrej et al. 2019) who observed no deleterious effects on the liver functions and immunity of broiler chickens fed diets supplemented with clove powder. Similarly, the serum antioxidative enzymes (catalase and glutathione peroxidase) were influenced by the supplementation of dietary SLM in the diet of the laying hen while superoxide dismutase was unaffected. Since (Delles et al. 2014) reported that catalase removes free radicals and other reactive species in the tissue to protect the body against oxidative stress, the increase in serum catalase and glutathione peroxidase indicated that SLM enhanced the secretion of more endogenous antioxidant enzymes in the laying birds. This report agreed with the findings of (Oloruntola et al. 2018) and (Daramola 2019) who also reported an increase in the concentration of catalase compared with the control group in broiler chickens fed herbal plants as additives.

Conclusions

The supplementation of SLM at 2.5% and 5.0% had no adverse effects on blood parameters as the birds were not anaemic and their immunity was not compromised. Likewise, the stimulatory effect of SLM on the serum antioxidative enzymes of layers revealed that its usage in layers production is highly beneficial but further research is required to investigate its implication on the egg formation processes in layers.

Abbreviations

SLM: *Syzygium aromaticum* leaf meal; NRC: National Research Council; SAS: Statistical analysis system; ESR: Erythrocyte sedimentation rate; PCV: Packed cell volume; MCV: Mean cell volume; Hb: Haemoglobin concentration; MCHC: Mean cell haemoglobin concentration; MCH: Mean cell haemoglobin.

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Author contributions

ISO did the writing of the manuscript. ADS participated in the feeding trial experiment, while OAA and FAG contributed to the design, work plan and implementation of the research work. All authors read and approved the final manuscript.

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Declarations

Ethical approval and consent to participation
All experimental procedures were approved by the Animal Welfare Committee of the Federal University of Technology, Akure after the due proposal has been presented and approved. The laying birds were sold off as spent birds a few weeks after completion of the study.

Consent for publication
Not applicable.

Competing interests
The author Report that there are no competing interests related to this study.

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